Subject: FW: CropLife Sediment Toxicology Project w/CLA Ecotox Working Group
Location: S12100

Start: Thu 4/28/2016 10:00 AM
End: Thu 4/28/2016 11:00 AM

Recurrence: (none)

Meeting Status: Accepted

Organizer: Pease, Anita

-----Original Appointment-----
From: Pease, Anita
Sent: Wednesday, April 27, 2016 8:39 AM
To: Pease, Anita; Cowles, James; kshenry@tkinet.com; Villanueva, Philip; Shamim, Mah; Sappington, Keith; Blankinship, Amy
Subject: CropLife Sediment Toxicology Project w/CLA Ecotox Working Group
When: Thursday, April 28, 2016 10:00 AM-11:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: S12100

As mentioned previously, CropLife America has a sediment tox subteam that has been compiling registrant data on chronic sediment tox studies conducted under the draft EPA guidelines or guidance. These studies are:

- Draft 850.1760 for the *Chironomus dilutus* chronic,
- Draft 850.1770 for the *Hyalella azteca* chronic
- 850.1780 for the *Leptocheirus plumulosus* chronic. No draft guideline exists for the *Leptocheirus* study, though a cross-walk from the EPA 600/R-01/020 manual is available.

Some info on this analysis was reported in a poster and oral presentation at SETAC last year. Updated versions of these are attached. Results of these reviews indicate that survival and growth, and for midges emergence, are generally the most sensitive endpoints, while reproduction is rarely (20% of studies for *H. azteca*; never for *C. dilutus*) the most sensitive. The data for *Leptocheirus* are less clear as the study method and success rate has been highly variable, though available data indicate that survival and growth are more sensitive than repro. Extensive work has been conducted recently on improving the reproducibility of the *Lepto* chronic method in consultation with EPA Duluth, USGS Colombia and the Army Corps lab in Vicksburg, MS. Few studies have yet been conducted with updated methods, though initial results are encouraging.

Would it be possible to meet with EFED staff to discuss the sediment data, the *Leptocheirus* method and other sediment tox topics?

SETAC
2015_CLA_SeaT...

SETAC
2015_CLA_Midg...

1

An evaluation of endpoint sensitivity for benthic invertebrate chronic toxicity tests

J Gates[1], T Valenti[2], M Cafarella[1], A Samel[3], B Sharma[4], H Krueger[5], J Staveley[6],
J Giddings[7], J Wang[8], M Bradley[9], S McGee[10], M McCoole[10], K Henry[11]

[1]Waterborne Environmental, Leesburg, VA; [2]Syngenta Crop Protection, Greensboro, NC; [3]DuPont, Wilmington, DE; [4]FMC Corporation, Ewing, NJ;
[5]Wildlife International, Easton, MD; [6]Exponent, Washington, DC; [7]Compliance Services International, Lakewood, WA; [8]BASF, Research Triangle Park, NC;
[9]Smithers Viscient, Wareham, MA; [10]Bayer CropScience, Research Triangle Park, NC; [11]Tessenderlo Kerley, Inc., Pheonix, AZ

Data Contributors: Syngenta Crop Protection, Bayer CropScience, Tessenderlo Kerley, BASF, AMVAC Chemical Corporation, Pyrethroid Working Group

Background

On October 26, 2007, sediment toxicity testing with benthic aquatic invertebrates became a conditional requirement as part of the Office of Pesticide Program's ecological effects data requirement contained in 40 CFR Part 158 Subpart G. This action led to efforts to improve the consistency of test performance and streamline chronic life-cycle test methods with benthic invertebrates. A focal area of discussion pertaining to these efforts included critically evaluating the relative sensitivities of required test endpoints within tests and among tests with different species as well as the utility of specific endpoints for defining biological thresholds of effect associated with contaminant exposure. To provide clarity in these pursuits, it is also important to consider variability within control responses as the value of monitoring some endpoints may be muted by reduced statistical power due to high variability (perhaps associated with natural biological variability). In addition to exploring relative endpoint sensitivity, determining possible data redundancy associated with endpoint overlap is also critical for improving confidence for defining effects thresholds based on these test endpoints and may also help manage laboratory resources. The Sediment Toxicology Subteam of CropLife America has compiled detailed data from chronic sediment toxicity tests conducted based on current USEPA draft test guidelines with *Chironomus dilutus*, *Hyalella azteca* and *Leptocheirus plumulosus*. This paper provides an overview of the analysis of control data for these species.

Data Summary

The table below presents the endpoints for each of the chronic sediment toxicity tests evaluated, along with the mean, standard deviation and coefficient of variation. The guideline control performance criteria are also provided.

Endpoint	Mean	Standard Deviation	Coefficient of Variation (%)*	Control Performance Criteria
Chironomus dilutus (n=16)				
Larval Survival (%)	87.2	8.94	0 – 44.6 (11.3)	≥ 70
Larval Growth (mg AFDM/individual)	1.43	0.424	1.81 – 60.5 (13.6)	≥ 0.48
Total Emergence (%)	71.7	8.15	4.94 – 60.6 (18.7)	≥ 50
Female Emergence Rate	0.0341	0.00576	3.04 – 18.1 (16.1)	
Male Emergence Rate	0.0366	0.00619	4.14 – 19.6 (10.6)	
Time to Oviposition (days)	1.35	0.218	11.0 – 100 (26.4)	
# Eggs/Mated Female	700	261	10.7 – 170 (38.7)	
# Egg Masses/Mated Female	0.710	0.179	8.79 – 161 (37.0)	
# Eggs/Egg Mass	805	341	0.0166 – 45.1 (20.2)	≥800[b]
Egg Hatchability (%)	90.5	5.41	0.303 – 45.0 (9.49)	≥80[b]
Female Days to Death (after emergence)	4.02	0.582	8.56 – 44.4 (19.8)	
Male Days to Death (after emergence)	3.60	0.526	10.0 – 45.7 (26.1)	
Hyalella azteca (n=14)				
28-Day Survival (%)	94.8	4.01	3.03 – 41.0 (8.84)	
35-Day Survival (%)	91.1	7.33	4.04 – 67.7 (12.6)	
42-Day Survival (%)	89.2	6.81	5.10 – 40.0 (12.3)	≥ 80
28-Day Length (mm)	5.18	0.762	0.681 – 13.4 (5.33)	
42-Day Length (mm)	5.68	0.345	2.25 – 9.52 (4.06)	≥ 3.2
35-Day Offspring/Female	5.81	2.43	18.9 – 113 (52.5)	
42-Day Offspring/Female	10.5	3.56	16.6 – 67.0 (44.8)	≥ 2 (28-42 days)
Male:Female Ratio	1.21	0.537	26.0 – 135 (76.0)	
Leptocheirus plumulosus				
Survival (%) (n=7)	85.3	6.73	3.19 – 17.4 (8.75)	≥ 80
Growth Rate (mg / amphipod/day) (n=7)	0.0592	0.0236	2.23 – 26.0 (13.3)	Measurable
# Offspring/Female (n=4)	6.06	4.19	11.1 – 95.2 (35.7)	Measurable

* Coefficient of variation (CV) is presented as the range of %CV values from individual studies, with the mean of the individual CV values presented in parentheses.
[b] Criteria for either number of eggs per egg mass or percent egg hatchability must be met.

Reference Guidelines And Methods
US EPA Draft Test Guidelines OPPTS 850 1760 (*Chironomus*), OPPTS 850 1770 (*Hyalella*). US EPA Test Methods 100 4 and 100 5 (*Chironomus* and *Hyalella*). Test Method 100 2 (*Leptocheirus*).

Comparison of Negative and Solvent Control

The test sediment used in each of these studies is either formulated (per OECD 218) or natural sediment. To ensure homogenous mixing of test material when spiking sediments, acetone is often used as a carrier solvent. Test material is dissolved in acetone and consistent volumes of solvent are mixed into a small mass of silica sand (2-10 g), after which the acetone is allowed to completely evaporate from the sand. The sand, coated with the test material is then mixed with the test sediment. While this resulting treatment is often referred to as the solvent control, no actual solvent is introduced into the exposure matrix and, as such, test organisms are never in contact with any solvent. Therefore, negative and solvent controls are functionally identical and presumably test organism performance should be consistent in each.

This conclusion is supported by study results as two-sample t-Tests (p ≤ 0.05) performed for each endpoint from individual studies indicate that there are only a few instances in which statistical differences were observed between solvent and negative controls (2.2% of all individual endpoint observations for *C. dilutus*, 4.5% for *H. azteca*, and 9.5% for *L. plumulosus*).

The figures below present the mean negative (N) and solvent (S) control data normalized to percentage of the overall N control mean for each endpoint. Different colors represent individual studies. When two-sample t-Tests (p ≤ 0.05) were conducted on the pooled dataset, no statistically significant differences were detected for any of the *C. dilutus*, *H. azteca* or *L. plumulosus* endpoints (p > 0.05). These results also strongly suggest that the occasional statistically significant difference between negative and solvent controls in individual studies is merely an artifact of natural biological variability.
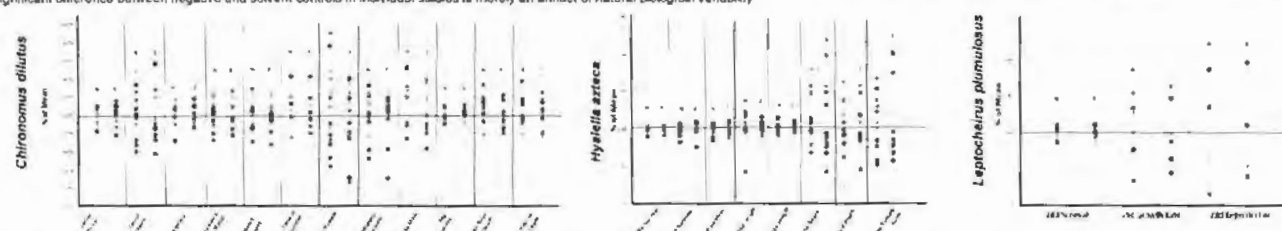
Relative Sensitivity of Endpoints

The figures below summarize the endpoints with greatest statistical sensitivity in each study. The y-axis indicates the ID number of each study and the circles represent endpoints within each study for which NOECs were derived. In some cases, a NOEC for only one endpoint was determined (e.g. *C. dilutus* study #1). In other cases, a NOEC could be determined for multiple endpoints. The endpoints derived are ranked based on sensitivity with red circles representing most sensitive endpoints and yellow representing less sensitive endpoints.
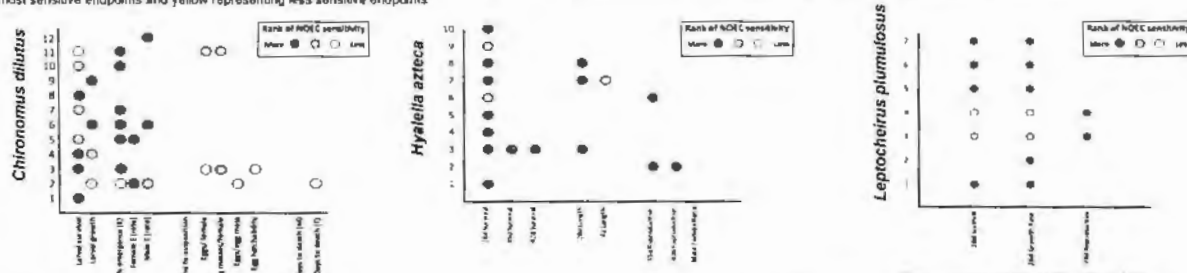
*Note: Reproduction was not evaluated in 3 of the 7 studies.

Key Findings

- **C. dilutus** Larval survival and growth, and emergence are the most statistically sensitive endpoints. Reproduction (5 endpoints) and time to adult death (2 endpoints) were never the most sensitive.
- **H. azteca** For the majority of studies (7 of 10), 28-day survival is the most statistically sensitive endpoint. Reproduction was most sensitive in only 20% of studies.
- **L. plumulosus** For the majority of studies (5 of 7), survival and growth rate are the most statistically sensitive endpoints. Additional data is required to assess relative sensitivity as some studies did not evaluate reproduction.
- High natural variability is associated with some parameters, which is likely reflected in the sensitivity of those endpoints.
- Performance is consistent between negative (N) and solvent controls (S), thus the use of two sets of control replicates may not provide value to these test methods.

Further Evaluations

By continuing to compile and analyze data, CLA aims to explore the following:

- Can the current study design be optimized?
  - Statistical power based on replication and variability
  - Gain in sensitivity versus resource expenditure
  - Do two sets of control treatments provide additional value?
- How do US chironomid endpoints compare to those from OECD 218 studies?
- Are there any trends when evaluating for the most sensitive species?

An evaluation of the relative sensitivity of endpoints generated during midge life-cycle sediment toxicity tests with pesticides as part of US FIFRA requirements.

Prepared by CropLife America Ecotox Working Group & Environmental Risk Assessment Committee-Sediment Discussion Group

SETAC North America, November 5, 2015

Presenter: Theodore Valenti – Syngenta Crop Protection, LLC
Contributers: Alan Samel - Dupont, Jane Staveley – Exponent, Michael Bradley – Smithers Viscient, Jiafan Wang - BASF, Maike Habekost - BASF, Hank Krueger – Wildlife International, Bibek Sharma - FMC, Jeff Giddings - CSI, Jennifer Gates – Waterborne, Matt Kern – Waterborne, Mark Cafarella – Waterborne, Sean McGee – Bayer CropScience, Kevin Henry – Tessenderlo Kerley , Inc.
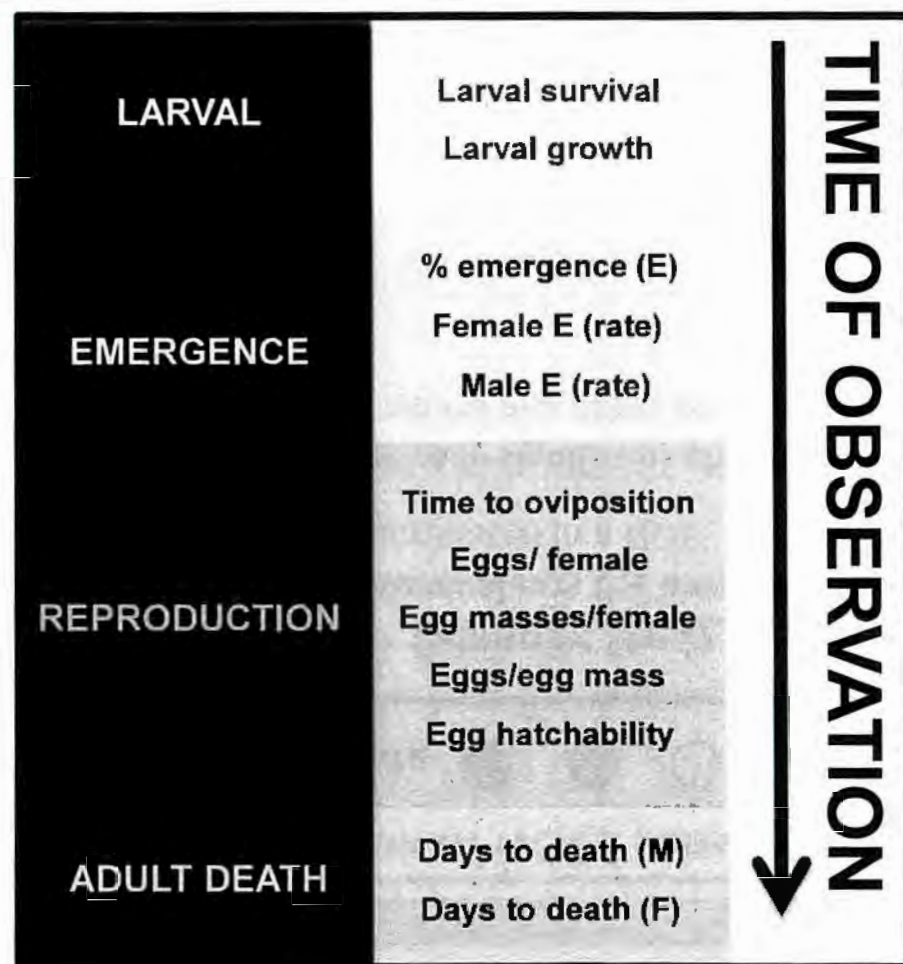
CROPLIFE AMERICA INITIATIVE
General overview

October 2012
Sediment toxicity testing with benthic invertebrates became a conditional FIFRA requirement (OPPTS 850.1760; *draft*)

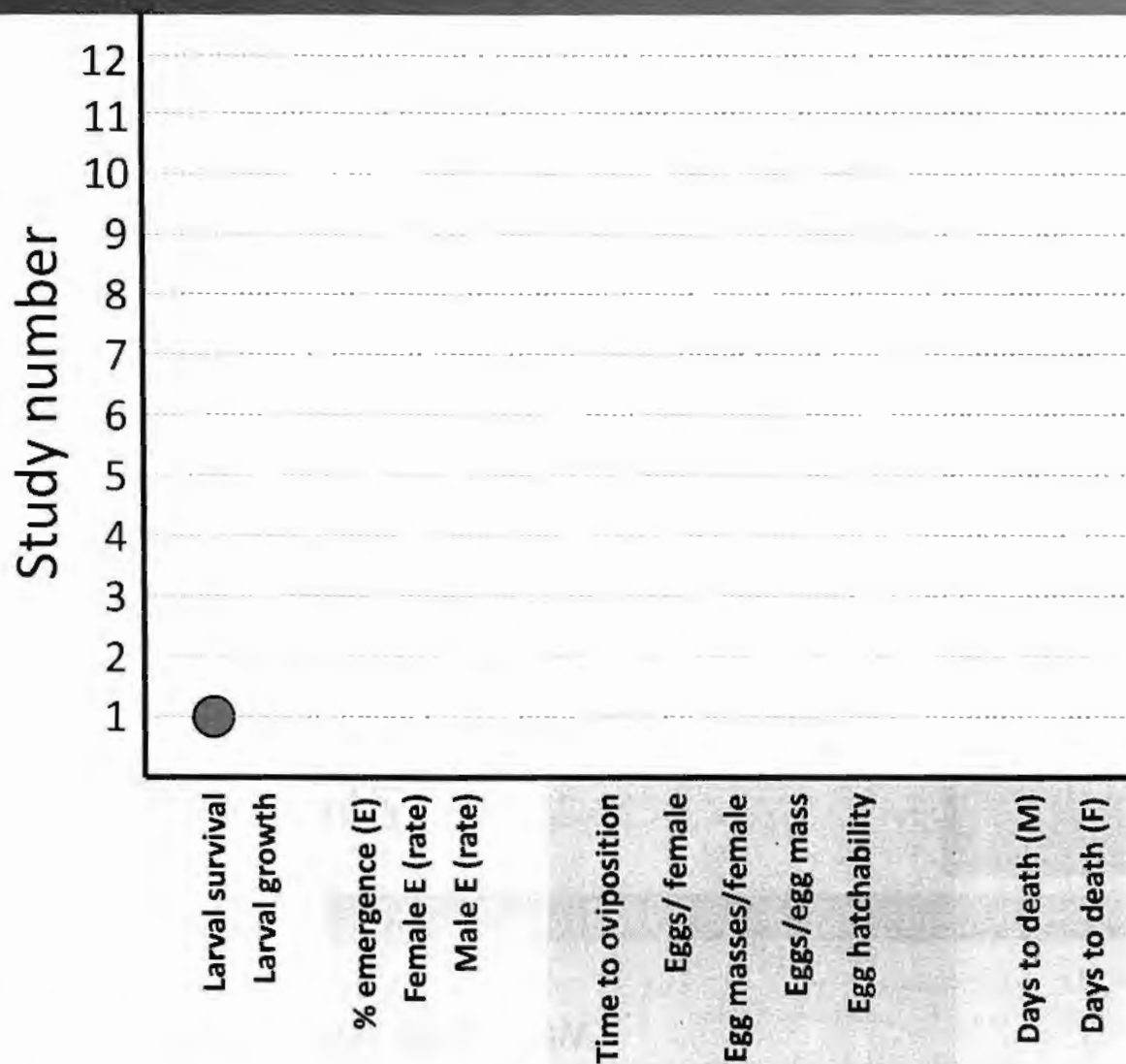Compiled data from 15 chronic *Chironomus dilutus* GLP studies

Completed analysis to examine:
1) Relative sensitivity of endpoints
2) Control performance

		TIME OF OBSERVATION
LARVAL	Larval survival Larval growth	
EMERGENCE	% emergence (E) Female E (rate) Male E (rate)	
REPRODUCTION	Time to oviposition Eggs/ female Egg masses/female Eggs/egg mass Egg hatchability	
ADULT DEATH	Days to death (M) Days to death (F)	

RELATIVE SENSITIVITY OF ENDPOINTS

Which endpoints drive the NOEC?

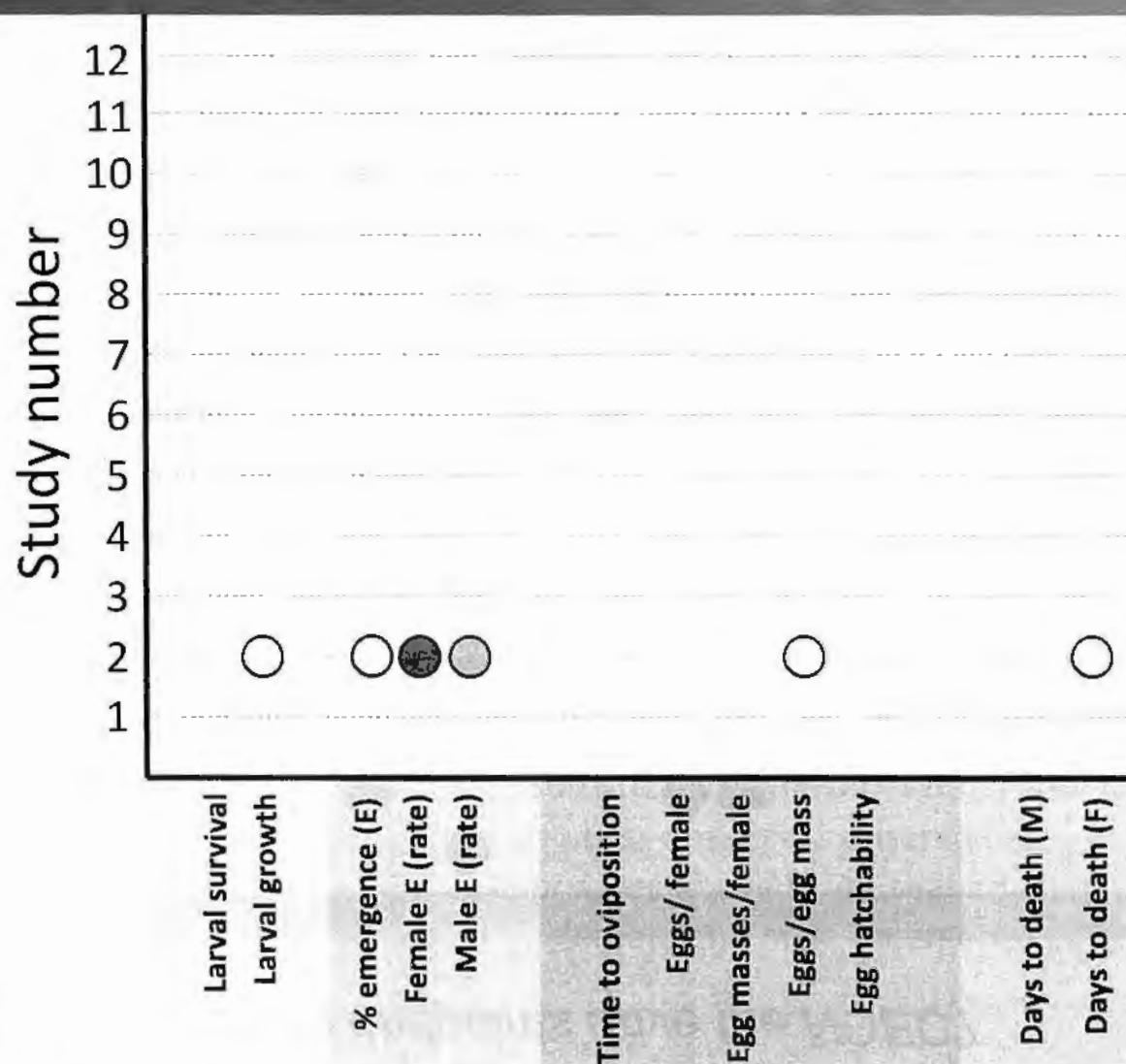**Rank of NOEC sensitivity**

More ● ◉ ○ Less

Example: Study 1

Larval survival was the only endpoint that culminated in a NOEC and LOEC

Statistically significant effects not noted for any other endpoint

RELATIVE SENSITIVITY OF ENDPOINTS

Which endpoints drive the NOEC?

Rank of NOEC sensitivity

More ● ◐ ○ Less

Example: Study 2

NOEC & LOEC determined for 6 endpoints

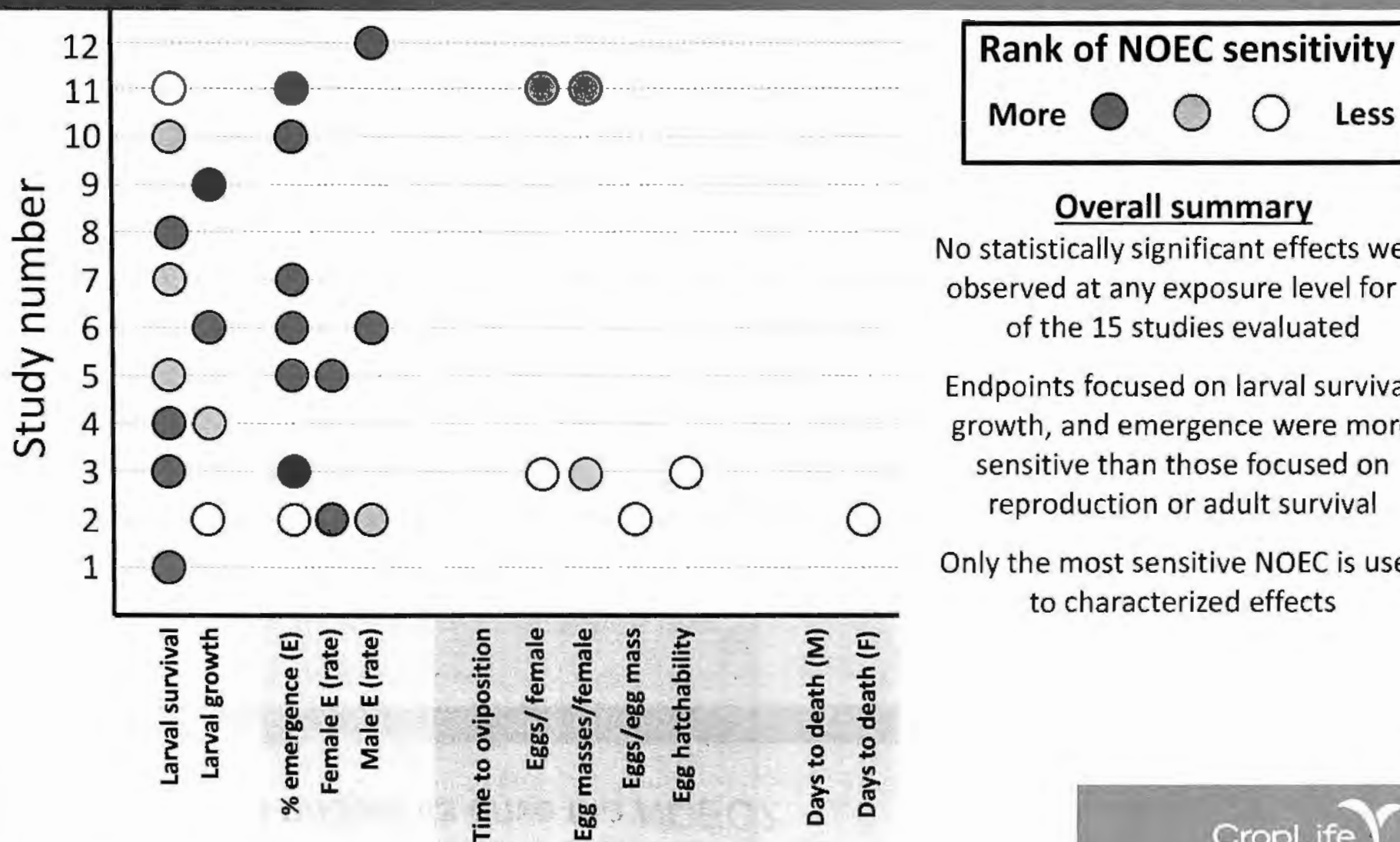Female emergence resulted in the lowest NOEC (most sensitive)

Significant effects on male emergence observed at a higher exposure level compared to female emergence (less sensitive NOEC)

Statistically significant effects for other endpoints observed at exposures above those that affected female and male emergence
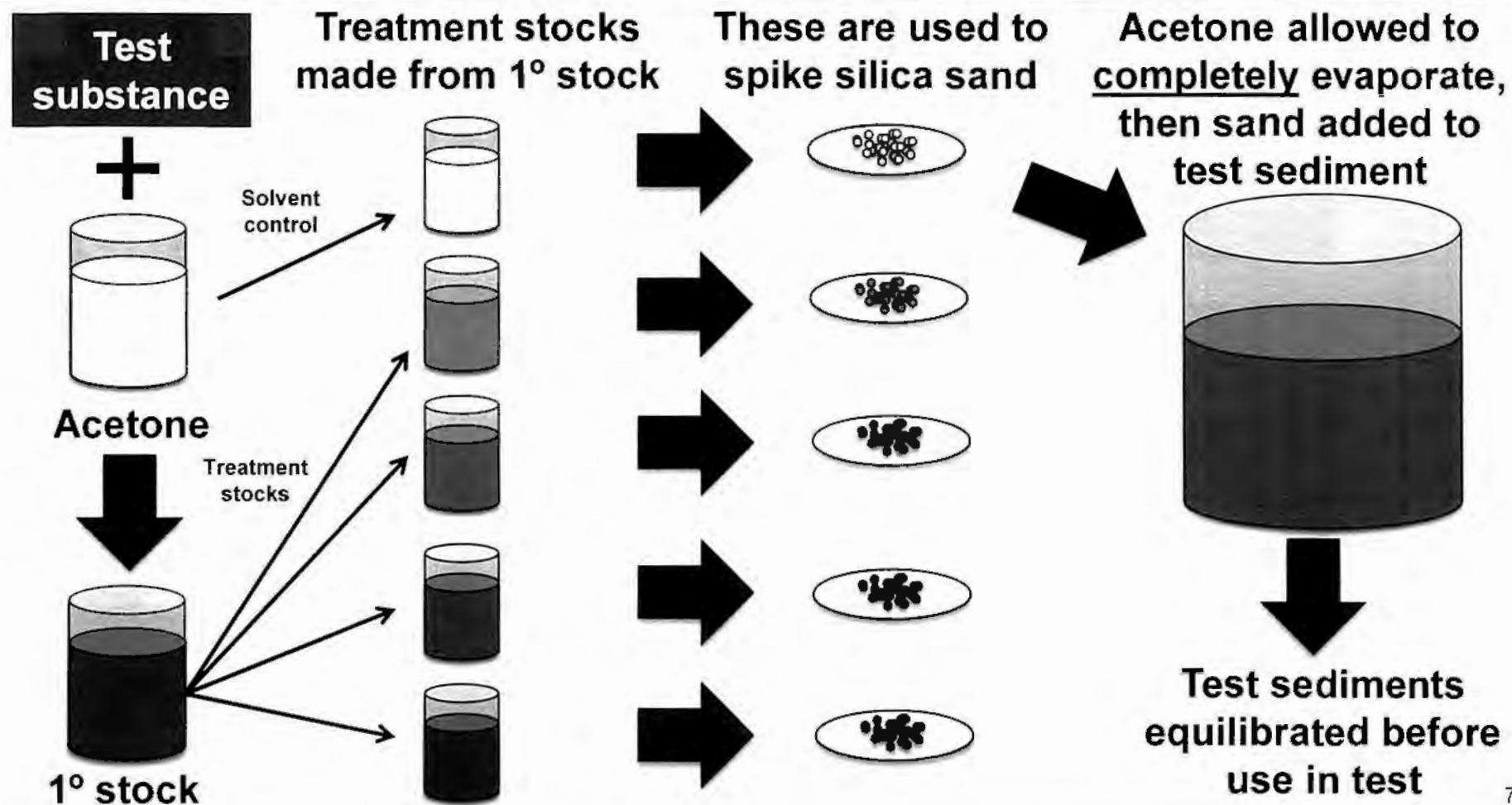
5

CropLife
✦AMERICA✦

RELATIVE SENSITIVITY OF ENDPOINTS

Which endpoints drive the NOEC?

Rank of NOEC sensitivity

More ● ◐ ○ Less

Overall summary

No statistically significant effects were observed at any exposure level for 3 of the 15 studies evaluated

Endpoints focused on larval survival, growth, and emergence were more sensitive than those focused on reproduction or adult survival

Only the most sensitive NOEC is used to characterized effects

6

NEGATIVE vs "SOLVENT" CONTROL

Negative and solvent controls are functionally identical
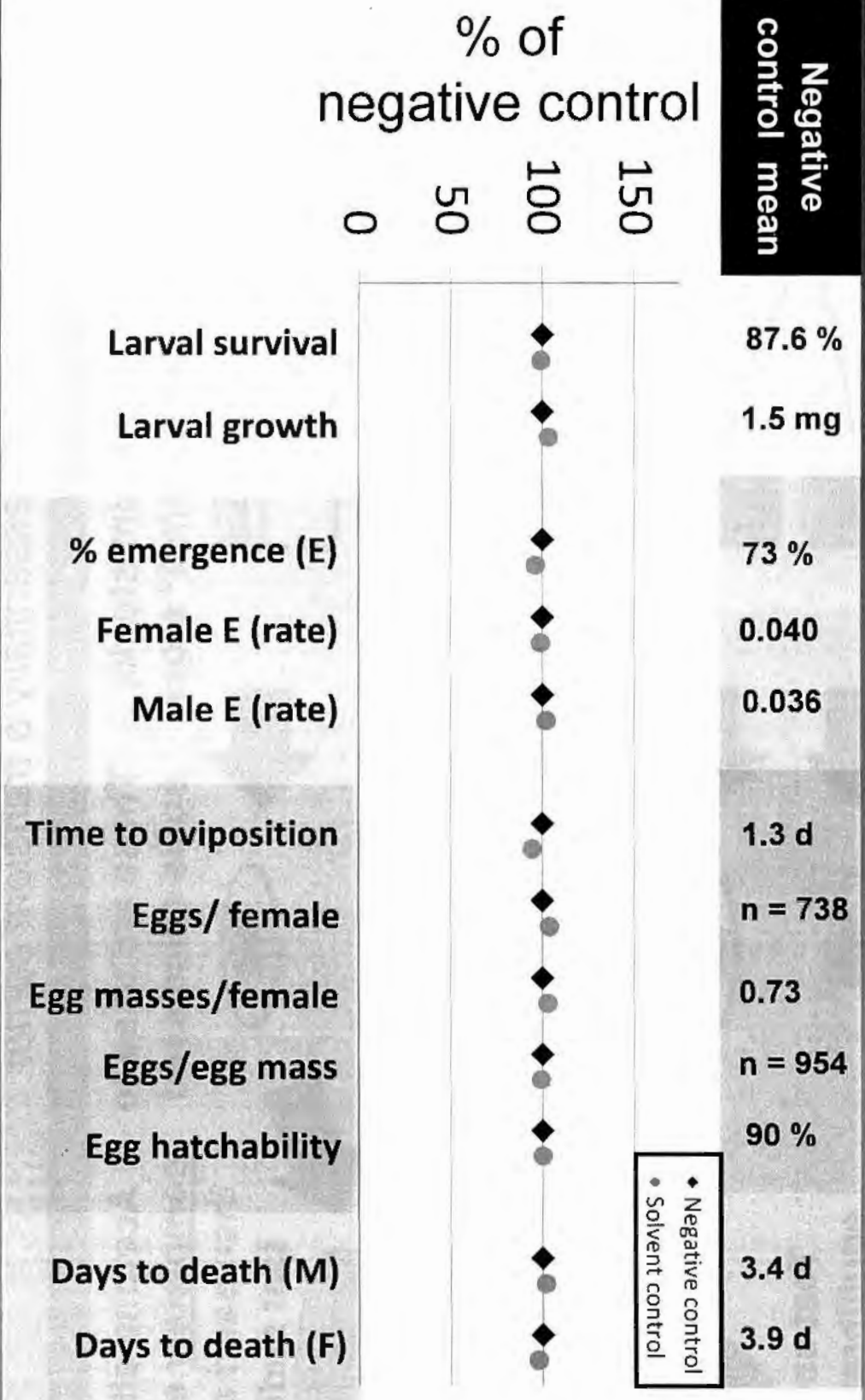*Solvent control essentially a transient carrier*

Test substance + **Acetone** → **1° stock**

Solvent control / **Treatment stocks**

Treatment stocks made from 1° stock → **These are used to spike silica sand** → **Acetone allowed to completely evaporate, then sand added to test sediment** → **Test sediments equilibrated before use in test**
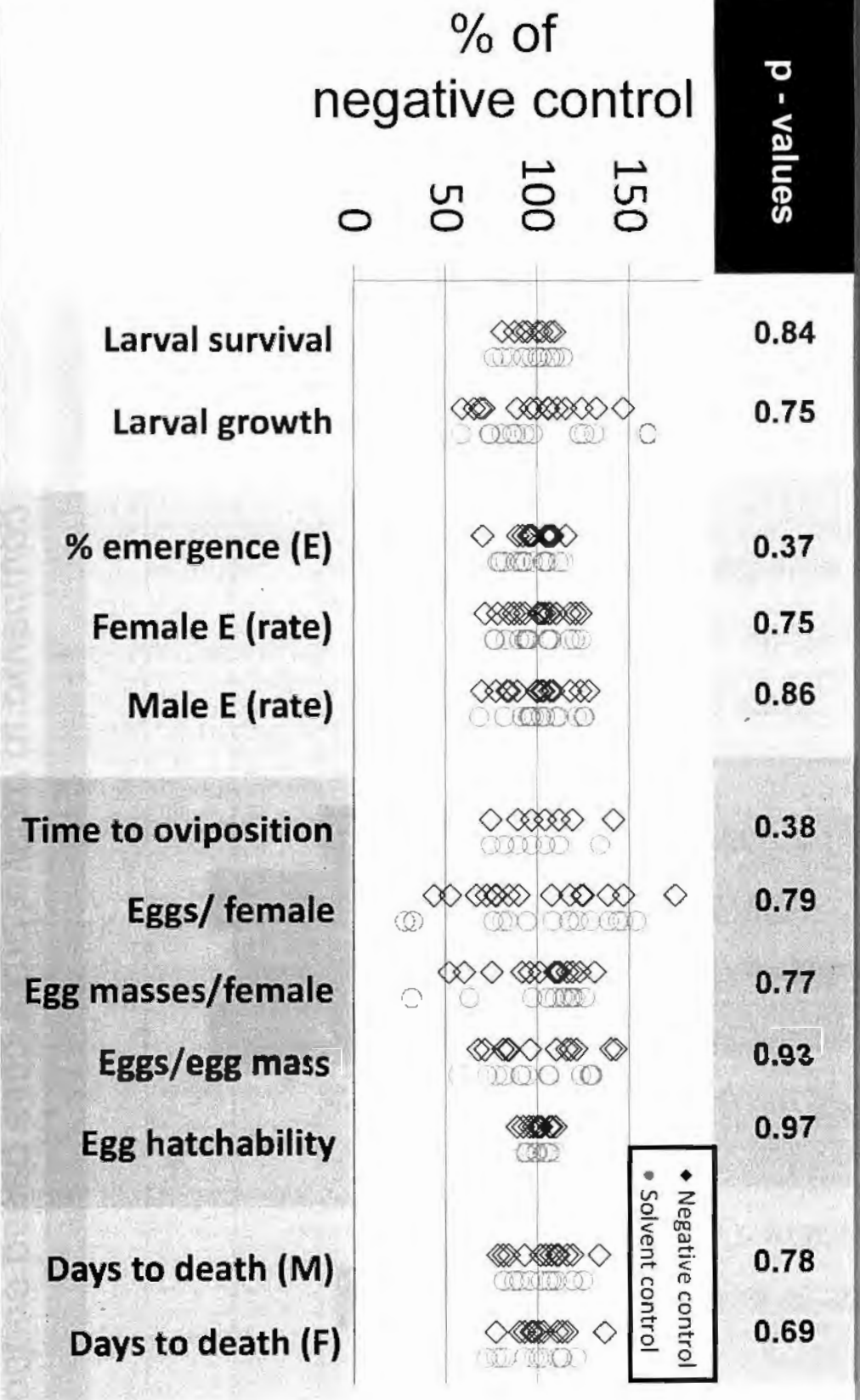
7

NEGATIVE vs "SOLVENT" CONTROL

As expected, performance between controls was essentially the same for all endpoints

	Negative control mean
Larval survival	87.6 %
Larval growth	1.5 mg
% emergence (E)	73 %
Female E (rate)	0.040
Male E (rate)	0.036
Time to oviposition	1.3 d
Eggs/ female	n = 738
Egg masses/female	0.73
Eggs/egg mass	n = 954
Egg hatchability	90 %
Days to death (M)	3.4 d
Days to death (F)	3.9 d

% of negative control: 0 50 100 150

◆ Negative control
● Solvent control

CropLife
AMERICA

NEGATIVE vs "SOLVENT" CONTROL

No statistically significant difference between pooled controls

Only 4 out of 180 within test comparisons differed statistically

% of negative control

Measure	p - values
Larval survival	0.84
Larval growth	0.75
% emergence (E)	0.37
Female E (rate)	0.75
Male E (rate)	0.86
Time to oviposition	0.38
Eggs/ female	0.79
Egg masses/female	0.77
Eggs/egg mass	0.93
Egg hatchability	0.97
Days to death (M)	0.78
Days to death (F)	0.69

Legend:
- ◆ Negative control
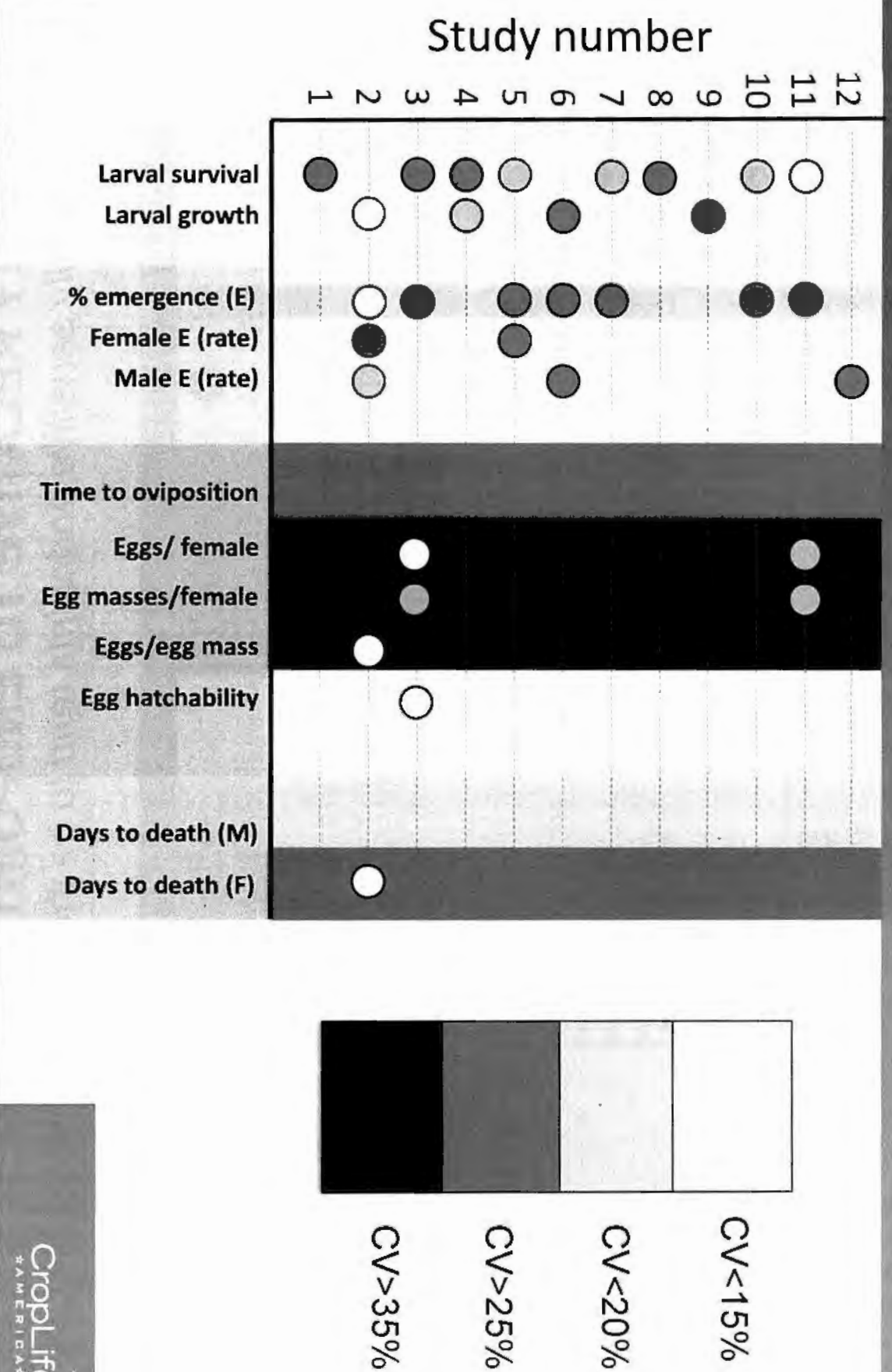- ○ Solvent control

Axis: 0, 50, 100, 150

9

ENDPOINT VARIABILITY

Coefficient of variation markedly lower for larval survival/growth and emergence compared to most reproductive based endpoints
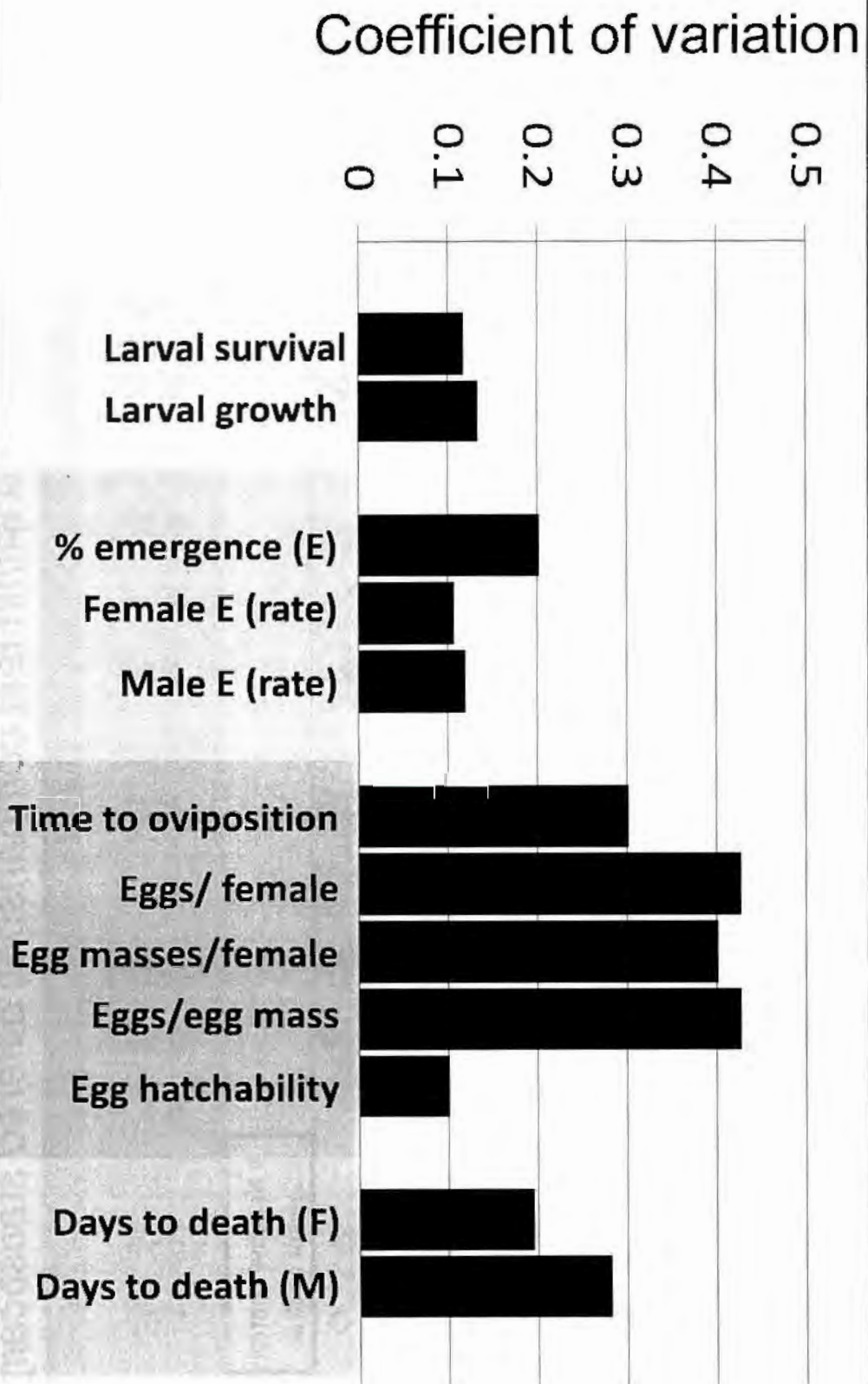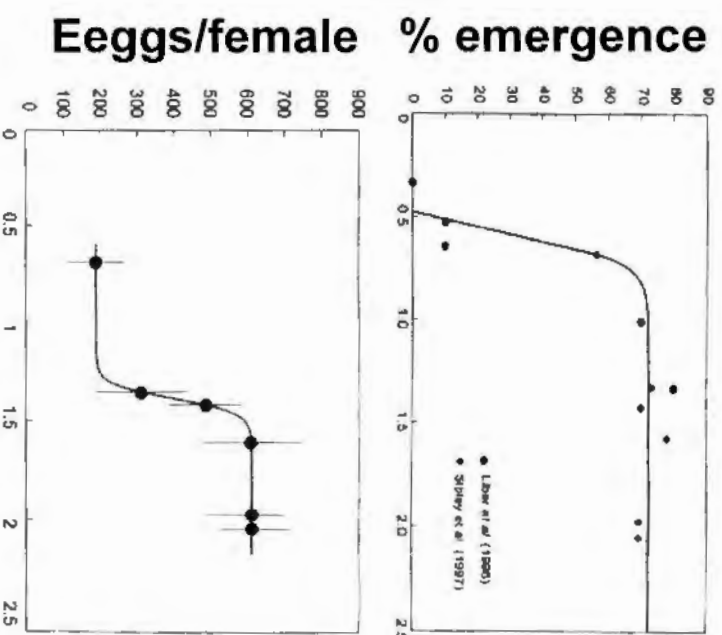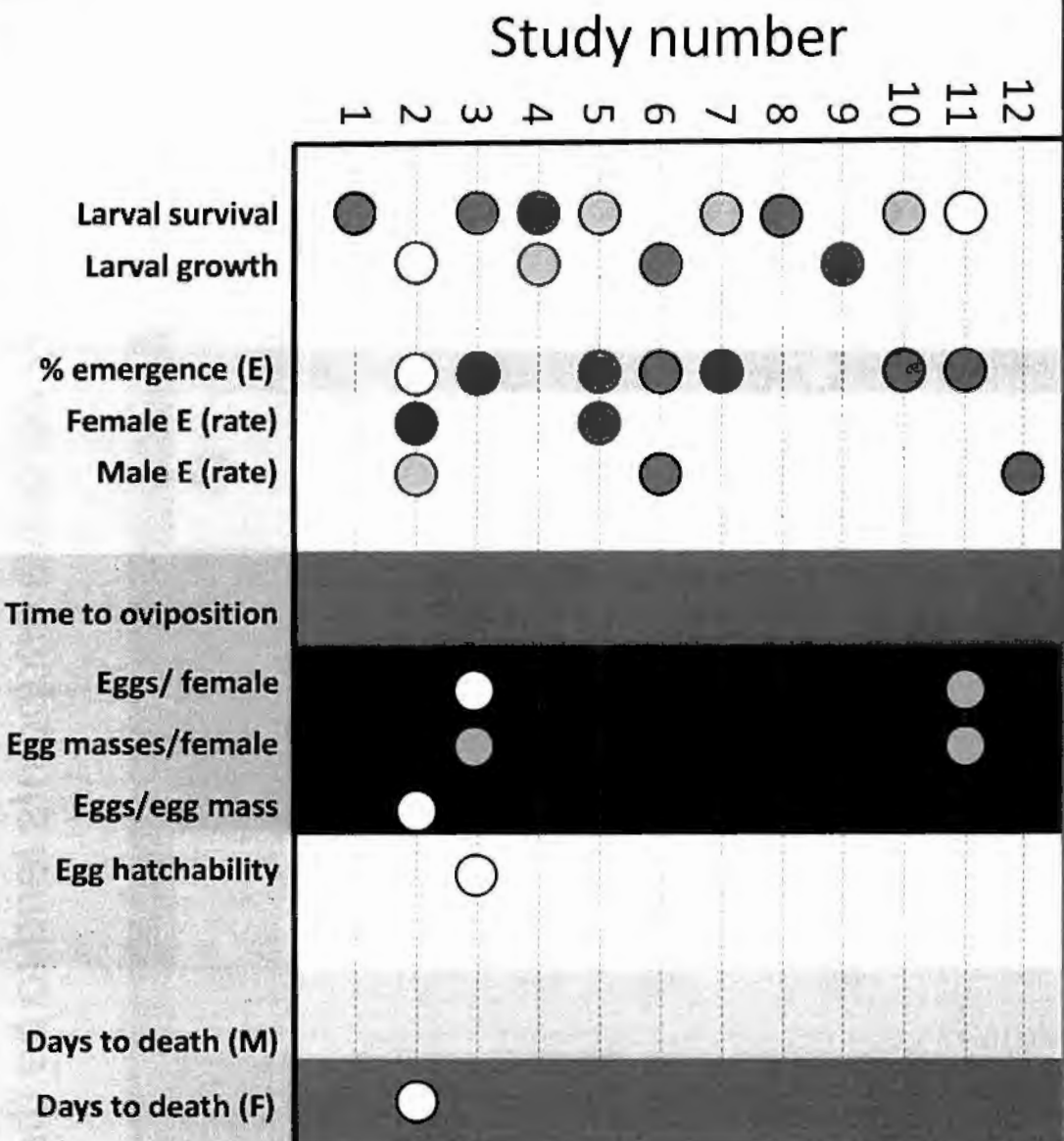
SENSITIVITY RELATIVE TO ENDPOINT VARIABILITY

Statistically sensitive endpoints tend to be those that have lower CVs

SENSITIVITY RELATIVE TO ENDPOINT VARIABILITY

Statistically sensitive endpoints tend to be those that have lower CVs

CONCLUSIONS

Major learnings and future considerations

Major Learnings

- Larval survival and growth, and adult emergence are statistically sensitive endpoints
- High natural variability associated with some endpoints (reproduction and adult survival)
- Performance consistent in negative and solvent controls

CONCLUSIONS

Major learnings and future considerations

Major Learnings

- Larval survival and growth, and adult emergence are statistically sensitive endpoints
- High natural variability associated with some endpoints (reproduction and adult survival)
- Performance consistent in negative and solvent controls

Future Considerations

- How might the current study design be optimized?
  - Focus on only a single control treatment
  - Refinement of monitored endpoints (Consider OECD 218/233)
  - Use of replicates (additional replicates not practical)
- Clear need to define ecologically relevant change

14

Acknowledgements

Entities that shared data:

CropLife America for providing support for data compilation and analysis.